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Receptor-mediated leukaemogenesis: hypothesis revisited

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Summary The discovery of the first example of retroviral transduction of an immunological effector molecule has led us to reconsider the possible importance of cell surface receptors of the immune system in leukaemia development. Antigen receptors on lymphoid cells not only bind external ligands but are crucial in the control of cellular proliferation. The concept of autocrine stimulation in oncogenesis is already well established and we see no reason to exclude the possibility of the analogous mechanism operating through antigen receptors. At present, we are investigating the oncogenic function of the retrovirus (FeLV-T17) carrying a T-cell receptor gene (v-*tcr*). In addressing the general concept of oncogenesis by ligand/receptor interactions in the immune system we face the problem of the diversity and, for T-cell antigen receptors, the complex nature of receptor-ligand interaction. Nevertheless, the wide implications of the model encourage us to continue to search for new experimental tools and approaches to the question.

It is around ten years since the publication of the receptor-mediated leukaemogenesis model by McGrath and Weissman (McGrath & Weissman, 1978, 1979). In essence, their hypothesis holds that retrovirus-induced tumours of lymphoid cells involve a mitogenic response arising from the binding of viral envelope proteins to immunological receptors on the tumour cell surface. The proliferating tumour cells, therefore, are clonally selected on the basis of their antigen receptor binding specificity. Despite its simplicity and intellectual attraction, a lack of supportive data from different laboratories has led to widespread scepticism regarding the model. However, the originators have maintained strong support for their hypothesis and have periodically presented supplementary evidence gleaned from a variety of retroviral systems (Weissman & McGrath, 1982; Weissman *et al.*, 1985; O'Neill *et al.*, 1987).

Our interest in the concept of receptor-mediated leukaemogenesis has been stimulated recently by our discovery of a retroviral provirus which carried the entire coding sequence of a T-cell antigen receptor β-chain (Figure 1, Fulton *et al.*, 1987). The retrovirus in question was found in a primary feline T-cell leukaemia where we had already noted the presence of a provirus carrying a v-*myc* gene. Thus, two separate host cell genes had apparently been transduced by FeLV in the genesis of a single naturally-occurring tumour. Since this observation was made in the Beatson Institute during the directorship of Dr John Paul, it is a pleasure to include a brief review of our findings and an updated interpretation of the work in a volume dedicated to Dr Paul's contributions to cancer research.

Discovery of tcr

For a number of years, we have studied feline leukaemia virus-associated tumours with the aim of elucidating the role of the virus and of host genetic targets in leukaemia development. In a proportion of tumours, we have found that the c-*myc* gene is affected, either by viral transduction or insertional mutagenesis (reviewed in Neil *et al.*, 1987*a*). Viruses carrying v-*myc* genes were found to be potently leukaemogenic, reproducing the thymic lymphosarcomas from which they were isolated, as early as twelve weeks after inoculation (Onions *et al.*, 1987). However, despite their relatively rapid onset, the tumours were oligoclonal or monoclonal and their characteristic phenotype (immortal, growth *in vitro* independent of exogenous interleukin-2) could not be reproduced *in vitro* by infection of thymocytes or bone marrow cells. These further observations led us to consider that at least one additional low frequency event may be required to complement the active v-*myc* gene *in vivo*. The most obvious course of action for us was to screen the tumours for rearrangements of known oncogene or T-cell growth factor/receptor loci which might mediate tumour progression. This approach has not proved fruitful so far,

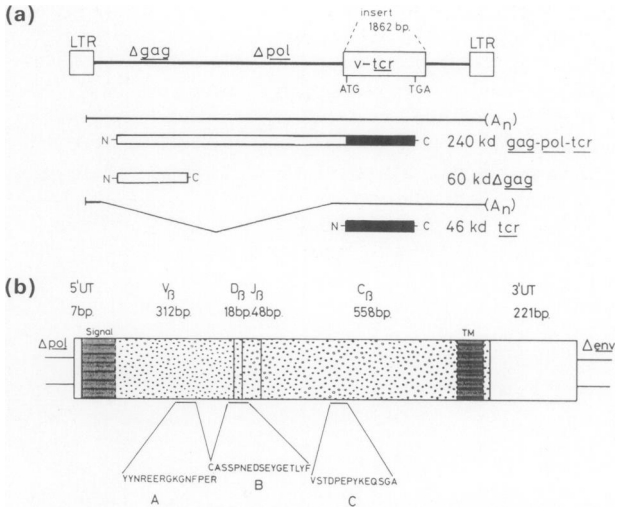

Figure 1 (a) Structure, expected transcripts and gene products from proviruses carrying the v-*tcr* gene. The proviruses have a typical FeLV proviral structure except that the 3′ end of *pol* and much of the *env* gene have been deleted and replaced by the *tcr* sequence. In this context a v-*tcr* product could be expressed either from full-length viral RNA or from a spliced subgenomic mRNA if the normal *env* splice acceptor site is utilised. These would lead, respectively, to a large read-through translation product linked to *gag-pol* sequences or to a discrete v-*tcr* product. Although the entire sequence of the proviruses carrying v-*tcr* have not been determined, we have recently been able to analyse their expression in transfected cells. A number of independent clones have been tested and all express a truncated form of the *gag* precursor protein (p60*gag*) but we have been unable to detect a read-through product large enough to encode the putative *gag-pol-tcr* read-through product. (b) Detailed structure of the host-derived v-*tcr* insert in FeLV proviruses from tumour T17. An entire reading frame for a T-cell antigen receptor β-chain product is present in place of the *env* gene and part of *pol*. The recombination points are (5′) immediately upstream of the first of two initiation codons for the β-chain product and (3′) at the consensus signal for polyadenylation of the β-chain gene transcript. Intron sequences are absent, as may be expected from the replication of this sequence as part of a retrovirus. However, all of the coding sequence elements expected in a mature, functionally rearranged β-chain gene are present. Marked under the sequence are the positions and amino acid sequence of peptides which have been used to raise antisera to the v-*tcr* product.

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although a more exhaustive search might be worthwhile in future. It was at this point, however, that we sought to define the differentiation state of FeLV v-*myc* induced and other tumours by assessing the state of rearrangement and expression of T-cell antigen receptor α and β-chain genes. The findings of this survey (Neil *et al.*, 1987*b*) showed that the tumours were heterogeneous except for those where the c-*myc* gene had been noticeably altered, either by proviral insertion at the locus or by the generation of a recombinant v-*myc*-containing FeLV. Most striking, however, was one case (T17) where the β-chain gene sequences were grossly amplified and aberrantly expressed. Subsequent analysis explained these unusual properties by the presence of multiple recombinant proviruses carrying the T-cell antigen receptor β-chain sequences which were integrated into tumour cell DNA.

Further studies: comparison of v-tcr *and cellular T-cell antigen receptor gene sequences*

On initial publication of our findings, we noted that alignment of the highly conserved Cβ elements of human and mouse with v-*tcr* revealed a potentially interesting mutational change – a substitution of lysine for methionine which was predicted from hydropathicity plots to be just inside the transmembrane region close to the cytoplasmic face. This was considered of some potential significance because of the precedent of the *neu* oncogene which can be activated by such a mechanism (Bargmann *et al.*, 1986) and because of the abundance of charged groups in the cognate regions of all the other T-cell antigen receptor gene products. However, subsequent analysis of the equivalent sequence from a feline Cβ gene revealed the same sequence as v-*tcr* (Figure 2), suggesting that this is a species-specific alteration which is unlikely to affect function of the T-cell receptor complex. Before ruling out the possible importance of the met/lys substitution, we should note that the Cβ gene which we analysed came from a tumour and not a germ-line DNA library. We have not yet performed a wider analysis of genes in the germ-line configuration.

Expression of the v-tcr *gene product*

Direct introduction of v-*tcr* constructs or proviruses carrying the gene into lymphoid cells has not yet been successful. However, transfection of heterologous cells (BHK 21 suspension cells) already containing FeLV-A/Glasgow-1 produced detectable levels of v-*tcr* viral RNA although virus titres cannot yet be measured satisfactorily since we have no transformation assay. Nevertheless, these virus harvests were the basis of the first *in vivo* experiments.

More recently, we have succeeded in stably transfecting v-*tcr* proviruses into a feline fibroblastic cell line, AH927. We have found that cell clones transfected with the v-*tcr* viruses express a truncated form of the major FeLV *gag* precursor protein Pr65*gag* which is relatively stable and does not appear to be packaged into virus particles. This truncated protein serves as a marker for expression of the v-*tcr* proviruses and was a prominent feature of the T17 tumour cell line. However, it is clear that the expression of this protein in AH927 fibroblast cells is at least two orders of magnitude lower than the Pr65*gag* of typical FeLV isolates such as FeLV-A/Glasgow-1. Examination of the LTR elements of the v-*tcr* provirus shows heterogeneity, since viruses have additional direct repeats including the putative enhancer sequences (R.F. and L. Holmes, unpublished results). It is perhaps significant that only the proviruses carrying the extra direct repeats shows detectable expression in fibroblastic cells. T-cell tropism directed by retroviral LTR elements has ample precedent (Celander & Haseltine, 1984; Chatis *et al.*, 1984; Desgroseillers & Jolicoeur, 1984; Short *et al.*, 1987) and would not be surprising in the case of the viruses we are studying, since these were cloned directly from T-cell tumour DNA. However, poor expression of the viruses in fibroblast cells present an obstacle to obtaining high titre stocks of the v-*tcr* viruses. We are at present

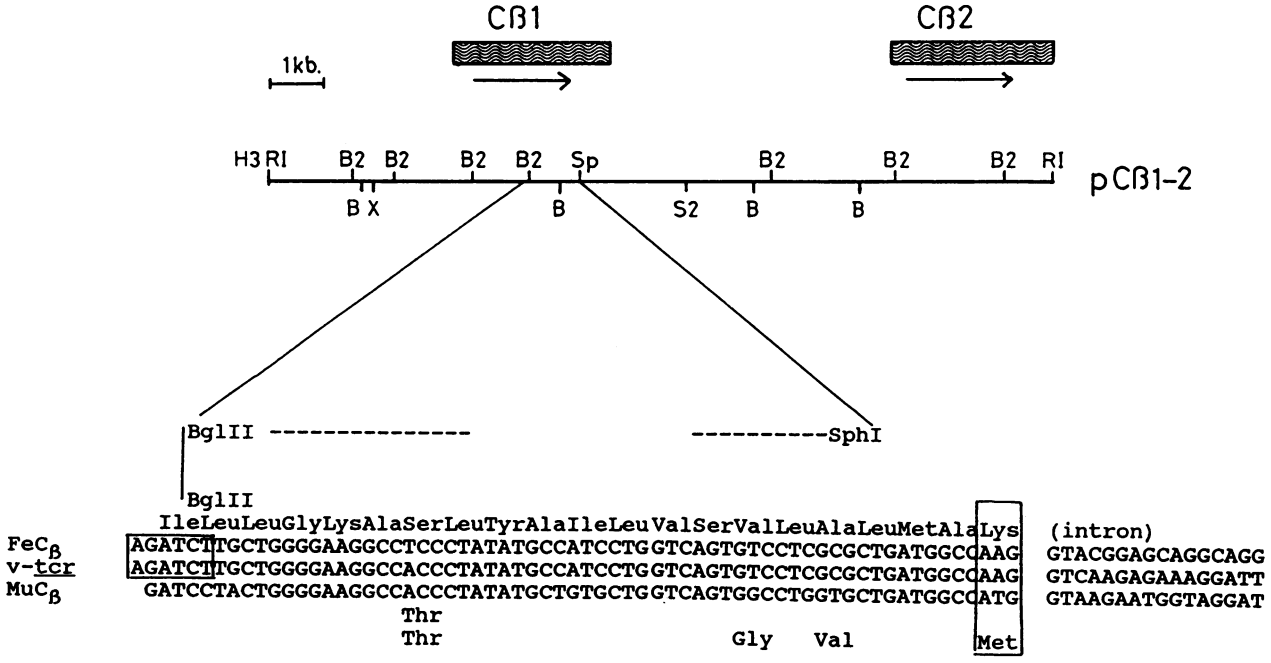

Figure 2 Sequence comparison of the feline Cβ gene with murine Cβ and v-*tcr*. The restriction enzyme cleavage map of a lambda clone of the feline Cβ region from the T17 tumour DNA library is shown at the top of the figure. The primary sequence illustrated includes a portion of Cβ exon sequence which encodes the transmembrane anchor domain of the protein product. We previously pointed out a difference in this domain which distinguished v-*tcr* from murine and human Cβ sequences (Fulton *et al.*, 1987) which was possibly of significance for v-*tcr* oncogenic function. However, as can be seen from the sequence figure, this change (met→lys) is also present in the feline Cβ gene. For this reason, we have revised our interpretation and we consider that the sequence difference is probably an evolutionary change of little functional significance although no functional studies have yet been performed.

exchanging the 5′ LTR of the v-*tcr* proviruses for the FeLV-A/Glasgow-1 LTR to circumvent this problem. With hindsight, it seems highly probable that poor expression in fibroblasts accounts for the weak or absent transforming activity of various FeLV/*myc* recominant viruses for feline fibroblasts (Neil *et al.*, 1984; Bonham *et al.*, 1987). Despite the high level expression of these proviruses in tumour DNA, transfection into feline AH927 fibroblast cells also gives very low level expression, and like the v-*tcr* proviruses, the v-*myc* viruses which have been transfected in our laboratory show LTR base sequence changes and direct repeats of enhancer sequences when compared to standard FeLV strains (M.S. and A.T., unpublished results; J. Casey, pers. comm.)

We have found no evidence as yet that the v-*tcr* gene is expressed as a *gag-pol-tcr* read-through product, although low levels of such a product cannot be ruled out. Possible expression of an intact, discrete v-*tcr* product from a spliced sub-genomic RNA is being addressed at present in the transfected fibroblast clones using anti-peptide antisera which are highly reactive with bacterial β-gal fusion proteins containing Vβ, VDJβ or Cβ sequences respectively (see Figure 1b). No positive results have yet been obtained. The antisera may not recognise the native product or, perhaps equally likely, the gene product is stable only in T-cells where it can associate with other components of the T-cell antigen receptor complex. A human T-cell receptor β-chain gene product has been found to be unstable when expressed in COS cells (C. Terhorst, pers. comm.).

It is likely that the level of T-cell antigen receptor complex at the cell surface is controlled under normal conditions by a requirement for stoichiometric balance of α, β and accessory CD3 chains. This is inferred from the normal composition of the cell surface complex and the nature of expression in the absence of one component, where cell surface transport does not occur (Weiss & Stobo, 1984). It is conceivable that the v-*tcr* product may be transported as a β:β homodimer or in aberrant complex with some other cell surface protein. This would provide a mechanism by which LTR-driven v-*tcr* expression could override the normal requirements for α:β stoichiometry. An intriguing observation by Weiss and co-workers is that Jurkat T-cell mutants which are mutant for β-chain expression are also depressed in transcription of the α gene. Transcription of α is restored upon transfection with a β-chain cDNA (Ohashi *et al.*, 1985). If transcription of α is regulated by the presence of the β-chain product itself, we can envisage a mechanism by which retrovirus-mediated overexpression could enhance the levels of T-cell antigen receptor at the cell surface without having to invoke any abnormality of transport, processing or stoichiometry.

Function of v-tcr

In vivo One experiment has already been performed in which a virus stock of a v-*tcr* virus (3–1) and FeLV-A/Glasgow-1 helper virus was inoculated into neonatal cats (D. Onions, unpublished results). Very recently, tumours have developed in these cats, some nine months after initiation of the experiment. One cat died with evident immunosuppressive disease which could have been attributable to the helper FeLV. Examination of the thymus and lymph node tissue from this animal revealed no evidence of the v-*tcr* provirus, although FeLV sequences were present and an unusually simple pattern of endogenous β-chain rearrangements was seen in each tissue. This may indicate the early stages of neoplastic disease or alternatively a severely restricted clonal repertoire due to an active immune response or loss of progenitor cells. Very recently, three cats have developed neoplastic disease, one myeloid leukaemia and two thymic lymphosarcomas. We are currently analysing the tissues to discover whether v-*tcr* sequences are present in the tumour DNA.

This experiment will be repeated with higher titre stocks of v-*tcr* virus and also with new constructs in which v-*tcr* and v-*myc* sequences have been linked on a single proviral structure.

In vitro Two alternative models for v-*tcr* oncogenic function are shown in Figure 3. These models are both based on the assumption that the transport and molecular association properties of the v-*tcr* product are essentially as for a normal β-chain. The first option presented is that v-*tcr* activates the antigen receptor complex in the absence of any external ligand. This may result from some intrinsic structural abnormality. We favoured this model when it appeared that v-*tcr* carried a mutation in the transmembrane domain, but as discussed earlier, it now appears that the sequence difference is species-specific rather than tumour-specific. A second possibility, which we now favour, is that v-*tcr* may be a high-affinity receptor for a ligand which was abundantly expressed during development of the tumour. If the ligand involved were the viral envelope glycoprotein, we would in essence have the McGrath/Weissman model as originally expounded. However, we would consider it equally possible that the ligand involved is some self or 'altered-self' antigen as discussed further below.

To address either model directly *in vitro*, we would ideally like to be able to introduce the v-*tcr* gene to mature feline T-cells and measure the results in terms of T-cell activation in the presence or absence of an antigen source (e.g. virus-infected or uninfected antigen presenting cells). The only available feline cell lines of mature phenotype are tumour

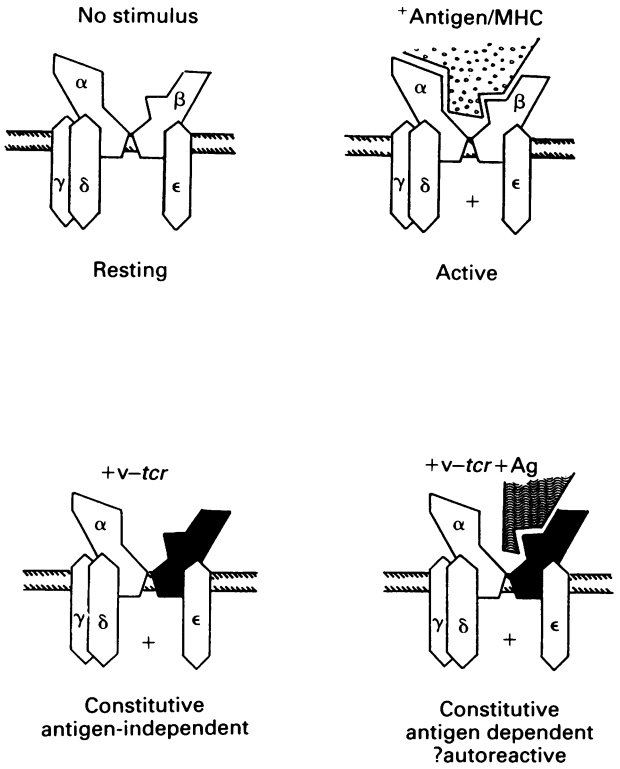

Figure 3 Models for the action of v-*tcr*. Based on the assumption that the important mode of expression of v-*tcr* is as a discrete gene product without fusion to other viral proteins, we envisage two distinct mechanisms by which it may disrupt the normal function of the antigen receptor complex. A schematic representation of the normal resting state of the antigen receptor and its interaction with foreign antigen in association with a self MHC molecule comprises the top half of the figure. In the lower half, a constitutive activation modcel is shown, whereby some structural anomaly of the v-*tcr* product itself confers a continually active state on the antigen receptor even in the absence of extracellular ligand. Alternatively, high affinity binding of the v-*tcr* product to some ubiquitous antigen may lead to much the same result in terms of driving cell proliferation. The nature of the ligand for v-*tcr* is not indicated but we speculate that high affinity for self MHC may suffice.

cell lines which are already infected with FeLV and furthermore contain FeLV/*myc* recombinant viruses. We are currently attempting to express the v-*tcr* gene in human T-cell lines, the most suitable of which appears to be a mutant Jurkat cell line which fails to express the β-chain of T-cell antigen receptor but which has all the other components necessary to reconstitute a functional complex (Weiss and Stobo, 1984). It is our hope that the v-*tcr* product will form a hybrid human:feline α:β heterodimer and that this may allow us to assess v-*tcr* function in human cells. It is encouraging to note that human:mouse heterodimers can be formed (Saito *et al.*, 1987). It must be recognised, however, that it may be difficult to reproduce antigen recognition and T-cell receptor function fully across species barriers.

Receptor-mediated leukaemogenesis: a reassessment

Arising from discussions in the last section, we propose a modified form of the receptor-mediated leukaemogenesis model in which the requirement for reactivity of the T-cell receptor with viral envelope antigen is discarded. We suggest instead that any high-affinity binding which activates the receptor may suffice. One of the theoretical difficulties arising from the original McGrath/Weissman model was that the viral envelope would have to mimic the structure of self MHC-antigen complexes. In our view, more credible ligands would be the MHC molecules themselves.

The role of v-myc Under normal circumstances, the poorly understood process of thymic education (Bevan, 1981) is thought to remove developing T-cells with excessive self-affinity. Any abnormal gene activation event which prevents clonal suppression or elimination during thymic education may allow the generation of autoreactive cells with an intrinsic drive to proliferate. We speculate that the c-*myc* gene product expressed under viral control may allow developing T-cells to circumvent the controls of the thymic deletion process. A relevant function of over-expressed c-*myc* in this respect may be the loss of response to growth inhibitory stimuli such as α and β type I interferon (Einat & Kimchi, 1988). In this way v-*myc* would provide an oncogenic signal complementing autoreactivity mediated by the antigen receptor.

The role of v-tcr In our view, the T17 tumour may be an exceptional case where the virus has captured the cell surface molecule conferring autoreactivity. Moreover, the presence of the v-*tcr* gene in a retrovirus may conceivably override transcriptional and maturation controls for the developing thymocyte, allowing high level expression of the TCR complex including the v-*tcr* product. The transduction of a β-chain gene, therefore, may be an unusually effective but not a necessary means of expressing a self-reactive T-cell receptor molecule. However, the role of v-*tcr* in our model is secondary to that of v-*myc* and we do not expect that v-*tcr* will necessarily have demonstrable *in vivo* oncogenicity in the absence of v-*myc* or in the absence of its proposed ligand (e.g. the appropriate self MHC). It is interesting to note that β-chain T-cell receptor gene products may be biased towards recognition of the MHC restriction element (Kappler *et al.*, 1987). The best opportunity to address our model appears to be in the mouse, since the T-cell receptor repertoire of inbred mice can now be analysed with sequence-specific gene probes or monoclonal antibodies (Chou *et al.*, 1987). Since autoreactivity via antigen receptors may conceivably apply to any malignancy of mature lymphoid cells, we are encouraged to persevere with viral model systems which may be manipulated both *in vitro* and *in vivo* to test this possibility.

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